A subset of neutrophil highly expressing CD49d and VEGFR1 can enhance pannus formation via increase FLS migration and invasion ability by up-regulating MMP3 and 13

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Background: Neutrophil is known to play an important role in the progression of rheumatoid arthritis (RA). Pannus formation requires hypoxia microenvironment and a small population of neutrophil highly expressing CD49d, VEGFR1 and CXCR4 is reported to induce angiogenesis at the sites of hypoxia[1,2].

Objectives: In the current study, we aim to identify this subset of neutrophil and investigate its function and role during the pannus formation.

Methods: Collagen-induced arthritis (CIA) model was applied in this study and CD49d+VEGFR1Ly6G+ neutrophil was monitored at the onset and remission of arthritis. The levels of IL-17, IL-4, IL-6, IL-10, TNF-a and IFN-g in CIA model sera were tested by ELISA at day 30, 36 and 42, respectively. mRNA expressions of cytokines including TNF-α, VEGF, IL-18 and MMP9 were detected by RT-PCR. Meanwhile, chemokines genes of CXCL10, CXCL9, CCL3 and CCL4 expressed in CD49d+VEGFR1 neutrophil were also measured. In vitro, synovial fibroblast-like cells (FLS) was co-cultured with MHL60, expressing CD49d and VEGFR1, and the migration and erosion was measured.

Results: CD49d+VEGFR1 neutrophil was detected in the peripheral blood and ankle at various time points with the peak on day 30. The gene expression of TNFα, VEGF, IL-18 as well as CXCL10, CXCL9, CCL3 and CCL4 was significantly increased in CD49d+VEGFR1 neutrophil. Pre-coculture with MHL60 was able to increase FLS migration and erosion. Meanwhile, the expression of MMP13, MMP3 was robustly enhanced at the mRNA and protein level.

Conclusions: A subset of neutrophil highly expressing CD49d and VEGFR1 was detected for the first time in CIA mice. CD49d+VEGFR1+ neutrophil is able to secrete various chemokine and cytokines. Meanwhile, it can enhance FLS migration and invasion ability via up-regulating MMP3 and 13.

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Effects of rituximab on the inflammatory and pro-thrombotic profiles of non-B cells of the immune and vascular systems of rheumatoid arthritis and systemic lupus erythematosus patients

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Background: Treatment with B-cell depletion, including rituximab (RTX), has been proven successful in achieving remission from the active disease in Rheumatoid Arthritis (RA) and Systemic Lupus Erythematosus (SLE) patients. However, the effect of RTX on the Cardiovascular disease (CVD) associated to both autoimmune conditions has not been elucidated yet. The aim of this study was to investigate whether B-cell depletion therapy with RTX has an impact on activation of non-B cells of the immune and vascular systems in the context of CVD in RA and SLE patients.
IMPACT OF OBESITY ON RHEUMATOID ARTHRITIS (RA) ANTIBODIES TO CITRULLINATED PROTEIN ANTIGENS

Methods: The analysis was carried out in plasma and purified leukocytes from 25 subjects, including 12 RA and 13 SLE patients. To evaluate the influence of B-cells depletion and ex vivo inflammatory profile of T-cells, purified lymphocytes from 6 RA and 7 SLE patients were treated with RTX (1 µg/ml) for 24 hours. B-cells depletion was assessed by flow cytometry and the changes occurred in the inflammatory profile of T-cells were analysed by RT-PCR. The changes promoted in the activity of key intracellular regulators of pro-inflammatory cytokines were analysed by western blot in proteins purified from lymphocytes. In a second set of experiments, supernatants from cultured lymphocytes of 6 RA and 7 SLE patients was added —either, in the presence or in the absence of RTX—to cultured endothelial cells (HUVECs), monocytes, and neutrophils isolated from Healthy Donors (HDs) and incubated for 6 hours. The changes induced in the inflammatory/pro-thrombotic profile of these cells was analysed by RT-PCR. Finally, serum obtained from 6 RA and 6 SLE patients at baseline and after 3 months of therapy with RTX, was added to HUVECs, monocytes, and neutrophils isolated from HDs and the response was analysed by RT-PCR.

Results: In parallel to the significant decline of B-cells, a downregulation of the pro-inflammatory profile of T-lymphocytes from RA and SLE patients was demonstrated, revealed by the significant drop of IL-1, IL-6, IL17, IFNγ, and TNFα gene expression levels. A decrease in the phosphorylation status and protein expression levels of STAT-3 and p38 was also found in T-cells treated with RTX. HUVECs, monocytes, and neutrophils incubated with the supernatant of RTX-treated lymphocytes from RA and SLE patients showed a decrease in the expression levels of various pro-thrombotic factors (i.e. TF, IL8, and VEGF) and cell-adhesion molecules (i.e. V-CAM, I-CAM and e-Selectin). Likewise, HUVECs, monocytes, and neutrophils treated with serum of RA and SLE patients after 3 months of therapy with RTX, showed a reduced expression of genes related to their pro-thrombotic and pro-inflammatory profile.

Conclusions: Depletion of B-cells induced by RTX might promote a beneficial effect in the CV risk-profile of RA and SLE patients through the modulation of the inflammatory and pro-thrombotic shapes of leukocytes and vascular endothelial cells.

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THU0075

IMPACT OF OBESITY ON RHEUMATOID ARTHRITIS (RA) ONSET AND PROGRESSION. IN VIVO AND IN VITRO EFFECTS OF SYNTHETIC DMARDS ON THE RA-ASSOCIATED METABOLIC ALTERATIONS


Objectives: 1) To evaluate the impact of obesity in RA onset and progression, 2) To analyse the in vivo effects of synthetic disease-modifying antirheumatic drugs (sDMARDs) on the obesity and IR in an obese collagen-induced arthritis (CIA) mouse model. 3) To study the in vitro effects of sDMARDs on the lipid and glucose homeostasis in adipose tissue (AT).

Methods: CIA was developed in obese and lean mice. 55 C57Bl/6 mice (4–5 weeks) were used. Forty-one mice were fed with high fat diet (60%) until reaching 8 months of therapy with RTX, showed a reduced expression of genes related to their pro-thrombotic and pro-inflammatory profile.

Conclusions: Depletion of B-cells induced by RTX might promote a beneficial effect in the CV risk-profile of RA and SLE patients through the modulation of the inflammatory and pro-thrombotic shapes of leukocytes and vascular endothelial cells.

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THU0076

ANTIBODIES TO CITRULLINATED PROTEIN ANTIGENS (ACPAs) IMPAIR ADIPOSE TISSUE DYSFUNCTION IMPAIRING ADIPOSECYTE DIFFERENTIATION, LIPID ACCUMULATION AND PROMOTING MACROPHAGE POLARIZATION. IN VITRO EFFECT OF BIOLOGIC DMARDS


Background: Adipose tissue (AT) dysfunction is an important determinant of inflammation- and lipid-induced metabolic complications. Rheumatoid arthritis (RA) is closely associated with metabolic comorbidities such as obesity or insulin resistance (IR). ACPAs are involved in the development of cardiovascular disease associated to this disorder. However, the role of ACPAs on the adipose tissue (AT) function is unravelled.

Objectives: 1) To analyse the direct effects of ACPAs on the AT function: adipocyte differentiation, macropolarisation, lip polynomial accumulation, and 2) To evaluate the effects tocolizumab (TCZ) or infliximab (IFX) on the metabolic alterations induced by ACPAs on AT.

Methods: IgGs-NHS (Normal Human Serum) and IgGs-ACPAs were isolated from serum of 20 controls and 20 RA patients. 3 T3-L1 fibroblasts were treated with IgGs-NHS or IgG-ACPAs alone or in combination with IFX or TCZ during several stages of the differentiation. At day 0 and day 9. Lipid accumulation was assessed by oil red O (ORO) staining. Monocytes, M0-macrophage and M1-macrophage THP-1 cells were treated with IgG-NHS or IgG-ACPAs alone for 12 hours or in combination with IFX and TCZ for another 12 hours. Macrophage polarisation was analysed by flow cytometry. Visceral and subcutaneous AT samples were obtained from 8 obese patients through bariatric surgery. AT samples were treated ex vivo with IgGs-NHS or IgG-ACPAs alone or in combination with biological DMARDs. Protein and gene expression of molecules involved in adipogenesis, inflammation, insulin signalling and lipid accumulation was analysed through RT-PCR, western blot and ELISA in all the experiments.

Results: In vitro treatment of M0 macrophages with IgG-ACPAs induced M1 polarisation state and impaired insulin signalling. 3 T3-L1 fibroblast treated with IgG-ACPAs at day 0 showed a impaired adipocyte differentiation shown by a reduction of genes involved in adipogenesis and lipid accumulation. Levels of accumulated lipids were also significantly reduced. Likewise, genes involved in insulin signalling were reduced. Treatment with IFX and TCZ after differentiation reverted the expression of these genes. At human adipose tissue level, the treatment with IgGs-ACPAs increased the levels of inflammatory markers, accompanied by a downregulation of genes involved in lipid accumulation, adipogenesis and insulin signalling. After treatment with biological DMARDs, inflammatory and metabolic alterations were reverted on human AT explants.

Conclusions: 1) ACPAs impairs AT function, acting in both, macrophages and adipocytes, inducing M1 macrophage polarisation and impairing adipogenesis and lipid accumulation in adipocytes, favouing an IR state. 2) TCZ and IFX might revert the metabolic alterations induced in AT by ACPAs. 3) Targeting these autoantibodies could be an excellent therapeutic strategy to restore AT function and reduce the metabolic complications related to RA.

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